

Figure 1 : Isolement de souche monocaryotique déficiente pour l'activité laccase

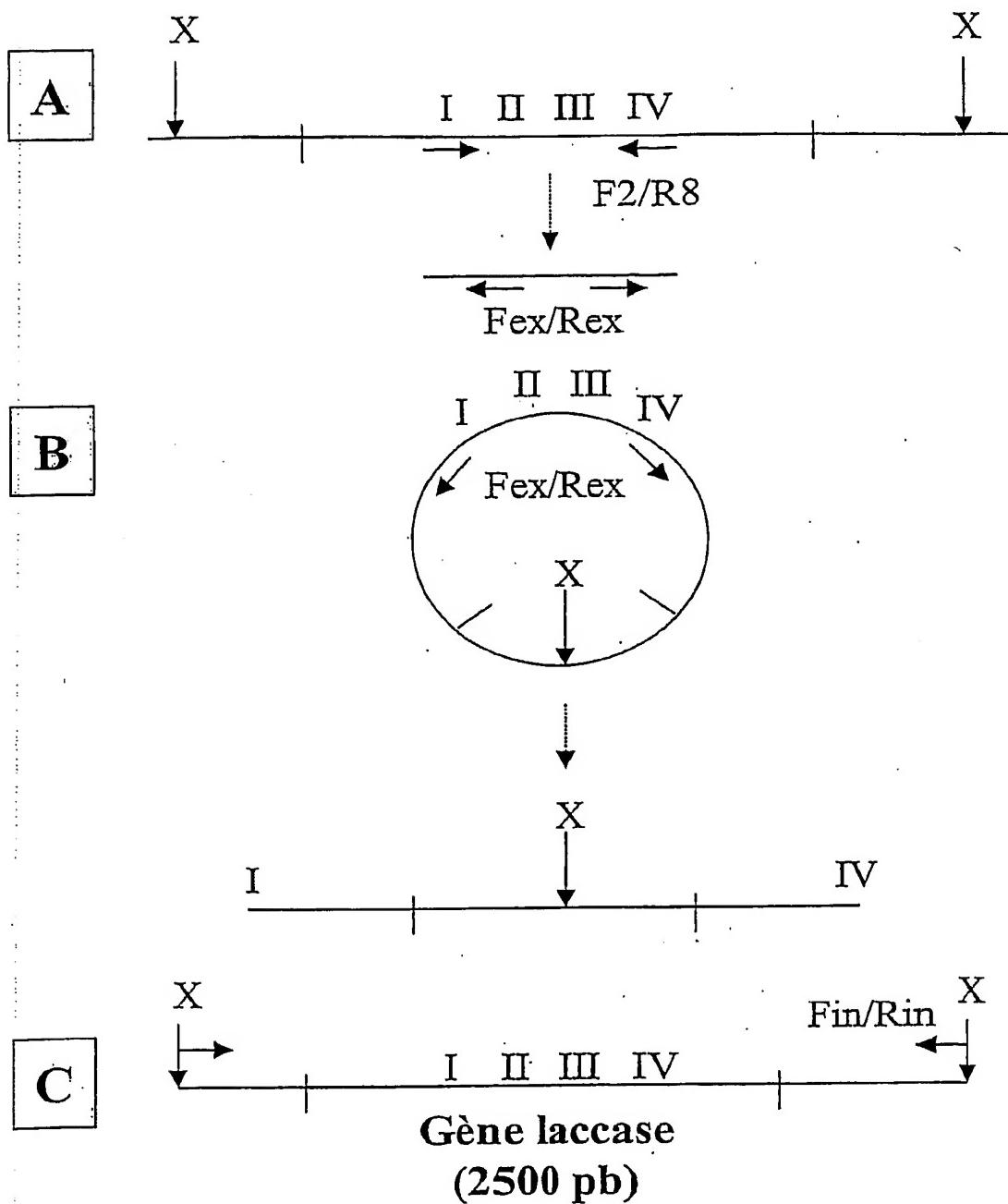


Figure 2 : Isolement du gène codant pour la laccase de *Pycnoporus cinnabarinus* laccase

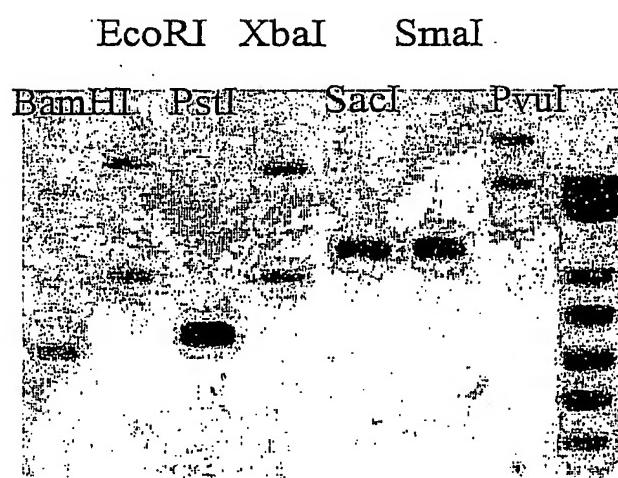


Figure 3 : Etude en Southern blot du gène codant pour la laccase de *Pynoporus cinnabarinus*

CTGCAGACATCTGGAGCGCCGTCTTCCCTAGTATAAATGATGTCGTCGCCAGGTCCTTGAGACCGCTCGAGTCCCACITGAGTTTAGGTAGGAC	100	
CTGTCCACCAAACCCCTCTTCTGATCATGTCGAGGTTCCAGTCCCTCTTCTGCTCCCTCACCGCTGGCCAACGCCAGCCATAGGGC	200	
M S R F Q S L F F F V L V S L T A V A N A A I G P	25	
CTGTGGCGACCTGACCCCTACCAATGCCAGGTCAGCCCCGATGGCTCGCTCCGAGGCCCTCGTGGTAACGGTATCACCCCTGCCCTCTCATCAC	300	
V A D L T L T N A Q V S P D G F A R E A V V V N G I T P A F L I T	58	
AGGCRAATAAGgtatgtatgtctgcgtccctcagactacatacatgtatccacaatcgtttagGGCGATCGATTCCAGCTCATGTCATGCCAG	400	
G N K G D R F Q L N V I D Q	72	
F2		
TTGACARATCATACCATGTTGAAACATCTAGTATTgtaaagggttcagttttccgactaccatgttattgaccatcaccactcgtag	500	
L T N H T M L K T S S I	H W H G	88
(I)		
CTTCTTCGAGCAAGGCACGAACCTGGGCCATGGTCCCGCTCGTGAACCAAGTGTCCCATCGCTTCGGGCCACTCGTTCTGTATGACTTCAGGTTCCC	600	
F F Q Q G T N W A D G P A F V N Q C P I A S G H S F L Y D F Q V P	121	
GACCAAGCAGgtacgaattccgtacacgtttcatgcgtcgcaactaaacctcttactagGGACTTTCTGGTACCATAGCCATCTCCACGCCAATA	700	
D Q A G T F W X H S H L S T Q Y	137	
(II)		
CTGCATGTTGAGGGGCCCTTCGTCGCTACGACCCCAACGATCCTCACCGTAGCTGTATGACATTGATAACGgtagcagatcatggatcgaa	800	
C D G L R G P F V V Y D P N D P H A S L Y D I D N D	163	
tattgcgtcacttatgttccctggatccagACGACACTGTCATTACGCTGGTGTGATGGTATCACGTCGGACCTCGCTTCCCgtac	900	
D T V I T L A D W Y H V A A K L G P R F P	184	
gtgtcaaatgtctacgagagatctcacatatacgactacttcgtcttattcagatTTGGCTCCGATTCAACCCCTATCATGGACTTGGTCGAA	1000	
F G S D S T L I N G L G R T	198	
CCACTGGCATAGCACCGTCGACTTGGCAGTTATCAAGGTCAACGGCAGGGCAACGGGgtatgtatggatgtatcgacatattggatgtatcatggc	1100	
T G I A P S D L A V I R V T Q G K R	216	
cttgcgttccacacgCTACCGCTTCGGCTGGTCTCGCTGGTCTTGCATCCGAAACCATACATTCAAGCATTGATAATCACAAATGACTATAATTGGCCGGA	1200	
Y R F R L V S L S C D B N H T F S I D N H T M T I E A D	245	
CTCGATCACACTCAACCCCTAGAGGTGATTCAATCCAGATTTTGCCGCAGCGCTACTCTTCGTTGgtagg tctgttagctctgtcatcaatgttgc	1300	
S I N T Q P L E V D S I O F A A Q R Y S F V	268	
cacagacatttttagatataccctttcaatgcagCTGGATGCTAGCCACGGTGGATAACTACTGGATCCGCACACCCCTGGCTTCGGAAACACAGGGT	1400	
L D A S Q P F V D N Y W I R A N P A F G N T G F	291	
TTGCTGGTGAATCAATTCTGCCATCTGGTGTATGATGGCCACCCGGAGATCGAGCCACTACGCTACGAGCCCTCTGAACCGAGGT	1500	
A G G I N S A I L R Y D G A P E I E P T S V Q T T P T K P L N E B V	324	
CGACTTGCATCCCTCTCGCCCTATGCCGTGtgatgttcaaagaacactcgatcaactaaatgtcatgtcaactcatatgttgcacacgCCTGGCAGC	1600	
D L H F P L S P M P V	P G S	337
CCCGAGCCCCGGAGGTGTCGACAAACCTCTGARCTTGGTCTTCACACTTCgtgatgtactggcgcgttccgttagcacacgttgcacaaaaggctgatccat	1700	
P E P G G V D K F L N L V F N F	353	
gcagAACGGCACCACCTTCTCATCAACGACCAACCTTGTCCCGCCGCTGTCCAGTCTGCTACAATCCTCAGTGGGGCGCAGGGGGCTCAGGAC	1800	
N G T H F I N D H T F V P P S V P V L L O I L S G A Q A A Q D	385	
CTGGTCCCGAGGGCAGGGTGTCTTCAGCACTCTCCATGTGAGATATCCTTCCCTGCCATGCCATGGATTCCCCCATCGTTCC	1900	
L V P E G S V F V L P S N S S I E I S F P A T A N A P G F P	H P F H	419
(III)		
ACTTGCACGGGtacgtctgccttccctcgatcaaaggcgagatgtactccatcacagCACGCCCTCGCTCGTCCGGAGCGCC GGGAGC	2000	
L H G H A F A V V R S A G S	433	
(IV)		
ACCGCTCTACAACTACGACAACCCGATCTCCGCACGTCAGCACGCCAGGCCAGCA ACCTCACGATTCGCTCGAGACCAATAACCCAGGGC	2100	
S V Y N Y D N P I F R D V V S T G Q P G D N V T I R F E T N N P G P	467	
R8		
CGTGGTCTCCACTGCCACATGACTTCCACCTCGACGCCAGGCTTGTGTAGTCATGCCAGGACACTCCGGACACCAAGGCCGAAAC CCTGTTCC	2200	
W E L H C H I D F H L D A G F A V V M A E D T P D T K A A N P V P	500	
(IV) (V) (VI)		
TCAGGCGTGGCGACTGTGCCCCATATGATGCACTTGACCCCAAGCGACCTCTGAGGGGATGTACTGTGACCTGGT GTGGGGGGACATGTCGA	2300	
Q A W S D L C P I Y D A L D P F S D L	518	
GGCTTTCTCATCGATCAGGGACTTCAGGTGGCAATAATACCTCACGGCTGGTGAATCGGAGACGCTGTGGCGTGGGTGTAACTCTGCTTGATGT	2400	
TGAAAAAAGGTTTATGTAGAACAACTTATGAGCAATCACGCAATAGGATTGTGTCGTTGAGCAATGCTTCTCCCTGACATTACTTTG	2500	
TGCGAGAAATGGGTCCATGAGACACATCATGAGCTCTCAATACCAAGAAGGATTACCCATGTCATACCAAGATCATGCTTCGCTGTCCGAATGG	2600	
TCTCATGTTGCGTTGAGCAGATCGCATGCTGAAAGGGATTAGTAT TACATGCAACATGCAACATTGAAAGGGGATGCAAGGGTCACTCGCG	2700	
TCAGTCGGCCAAGTAGCGACCTTGGCGACTGCCGTGTTAACCTGAGCTATGCTTCAGAACTCCGCGTATCGAGAGCGATCGTGTACGTTCCGGGAT	2800	
AGATCCATTGATCCCCGCTGGTGGCGCTGGATGGCCCGAGCGTCACCGGAGCTTCGCGATCCGCTTTCTAGGGGAGGGCGTGTACCCCG	2900	
CGTGTACGAGACGAGCTGCTGGTGGGGCGAAGGGCCGAAGGAGCCACTCACGAAGAGCAATGCGACGTAATCCGAGGTAGCCTTGCCGTTA	3000	
GTCACACGCACGGAGAACGTGTCGAGCGCCGAGGTGAGGAGGGCGCTTCTGACCGCGCTGTACGAGGTGCGAAATCGAAATACGTCGATGGCG	3100	
GTCCCTCCRAAGTCCGTGACGTTGGTCGCATCGGCCGCCCTGGAGCTGCCAAGAGAAATCGAAGGGTGTGAAGTGCAGTCCAAAGCCAATTGCTA	3200	
GACCGGGCGTGGCGGTGTACCACTTGTATGACGCCGGGTTGACGCGCTTGGCGAAGGGT CATGTCAGTCATGCCACCTGATCGCGTAGATGGCG	3300	
GGGTATTGGGTGATGGCAGGGCGTCTGCA	3331	

Figure 4 : Séquence du gène codant pour la laccase de *Pycnoporus cinnabarinus*

AGATCTCCGAACCAGAAATGCGATTGCGTTAGGCCAATTAAGAATAAAGCTGCGTCAGGCAGCGACGTA
TCTTGATCCCATCATTGACTCACCGGCATCGCGTCAACACCAAGCAAGCTCGTCCCACCCATAGGCCTGCA
CCGGCCGGCGTGCCTGAGGTACATGAGCGGGCGAAAGTCCGCATTGGTAGCCCTGTCGTGGACGCG
CGGCGATGAAACGTTCCCACCATGGGAAGAAACGTCGCGGCCATCATCCCTCACCGGATGACAAGGC
GGCGTGCCTGCGGCCATTGGCGAGAGCCGGCGACATGCACAGCGAAGGTCGTTGCGATGGGAAGCAGG
CAATCAGTGGGTGCTTACGCCACGATGGTGGGGAGCGTAGGCGCCCTCCATAAGGCGGCAAGCATH
ATGATGCTCTCCGATTGGGAAGCCTGGTGGATGCTGGAGAGACTCTCTCCAGAGACCGAGTGTGCGCAAC
GTTCCCTGGCTTGGAAAGACTTTAAAGTGAGTGAGAAGGGCGAGCAGGGACGATCATCGATTGCAAGG
ATCGGCATCCCTGCCAGGGAGGATGGCTTGGTAGACATTCGCGGAAGGTTCTAGATGAGCAGGGC
TTCTTGGATGATCATGCTGAACTTTTCTGACCTCGTGGTAGCGATGGCAGGATTGAGCATTACGGT
ATGCCCTCCATTCAAACGATAACCCCTTCCCTGCCAGGGTAGGGTGGATGCTGGTCAAGGAAACGACTTGT
CCTAGGCTATTACACCCCTCGAACATCCCTATTACGGTGTCTGTAAGGAAACGACTTGT
ACATGAAGTGCAGCATACTGTCGCCGGTCTCGCAGTACAGCGCTAGTACGGGAAGTCGACATCCAAGCGT
TCAGTCACCACATGGCAAAAAAGCTGCACCATACTCTTATGGTAGGTTCTGAGTGGTATAACAGTCAT
TCATGAGGGAAATGCCAACCGGATAGGGTGTGGCGGCCAATATTACATGCCCTGGCAATAGTCGATGT
CCTTGTCAATGAATATCATGGTCACATGTGGAGACGGTTAACACAGCGTACTGTGAATCCCTGGTGT
GTTGGGCCAACAGGTACGTTGCAGGAACACCAATATCTTCCGGCAGGCCAGTTCTTGCAGGCCACAG
GCAGGCATCGCIAACAGATCCCAGCCATCCGGCTCTGACATTGGGATACCTGAAGCCCTTCAGGTACGG
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GTGAAAGGCTCCATCATAGCGGCTCAACTCTACCTCGAACACAGCGGGAAACTTATTATG
TGGACAAGGCCGAGCTATGATAGCTGCTCCGAAGTGGTAGTCCCACGTTCTGGCAGGAAACAGT
CTCGGAAAAATAAGAAGAATATTGAGGTGCGTAGGGCTATGCCCAAATGCGCACACACGGAGGCTT
GGAGATGAAGCGCCCGTAGCGGTAAGGGAGTTGGTCAACGCCGCCCCGACGACTCTCTCTTCCAG
CATCATGTCGGCGCAAACCTTACCCCTATTGACCAACTCCACGAGAAAGCAGGAACAGCTTCCCTGT
CTCATGACGTCCGCAATCCAGACCCCTAGCCGGTTCTACTCATCGTTATCCCTGCCGCAATGGTAGT
GTCAGCCTGGCCAGTGCCTAGCCGCTCTTGCTGCACTAGAGAAGCCCATGAGACAGCGTTTTGC
TTTATTCTGCTTTCTACGGCTGAATCGGCTGCACGGCAGATAAAATCGGCCGGAAATGCTATAGCC
CATAGCCCGTATGAGATCGCAAAAGGCTTGTCACTCAGGTCGGCGAGTGGCTCTCACGAAGAGCGTCAA
CTTCGCGCAGCGCCCTTCAGGGCAAGATAGATCTCCCATCATCCCCTACTGCGCTCAGCGCCGGTAC
CGAACAAATTGACTTACCGACATCCCTGGGACGCGCAAATGCTGTGACGGAACGTAATCCTCTCGT
GCCCTTTGCTCTACGCATTCCGTGTGGTCTGCGCAGCGCCGCTCATCAGGACCAGCAGTCTCAAT
GCTGGTACCGGACAATGGTGACACTGCGGCAACTGAGTAGGTCGGTCACTCTGGTGCACCGTGCCTAC
GCTGACCTCGGGATACTGTCCTGCAGACATCTGGAGCGCCTGTCTTCCCTAGTATAATGATGTC
CGCAGGTCTTGAAGACCGCTCGAGTCCCACTTGAGTTAGGTAGGACCTGTCCACCAAACCCCTTTCT
GATCATG

Figure 5 : Séquence de la séquence promotrice du gène codant pour la laccase de *Pycnoporus cinnabarinus* (jusqu'à l'ATG codant pour la méthionine de la laccase)

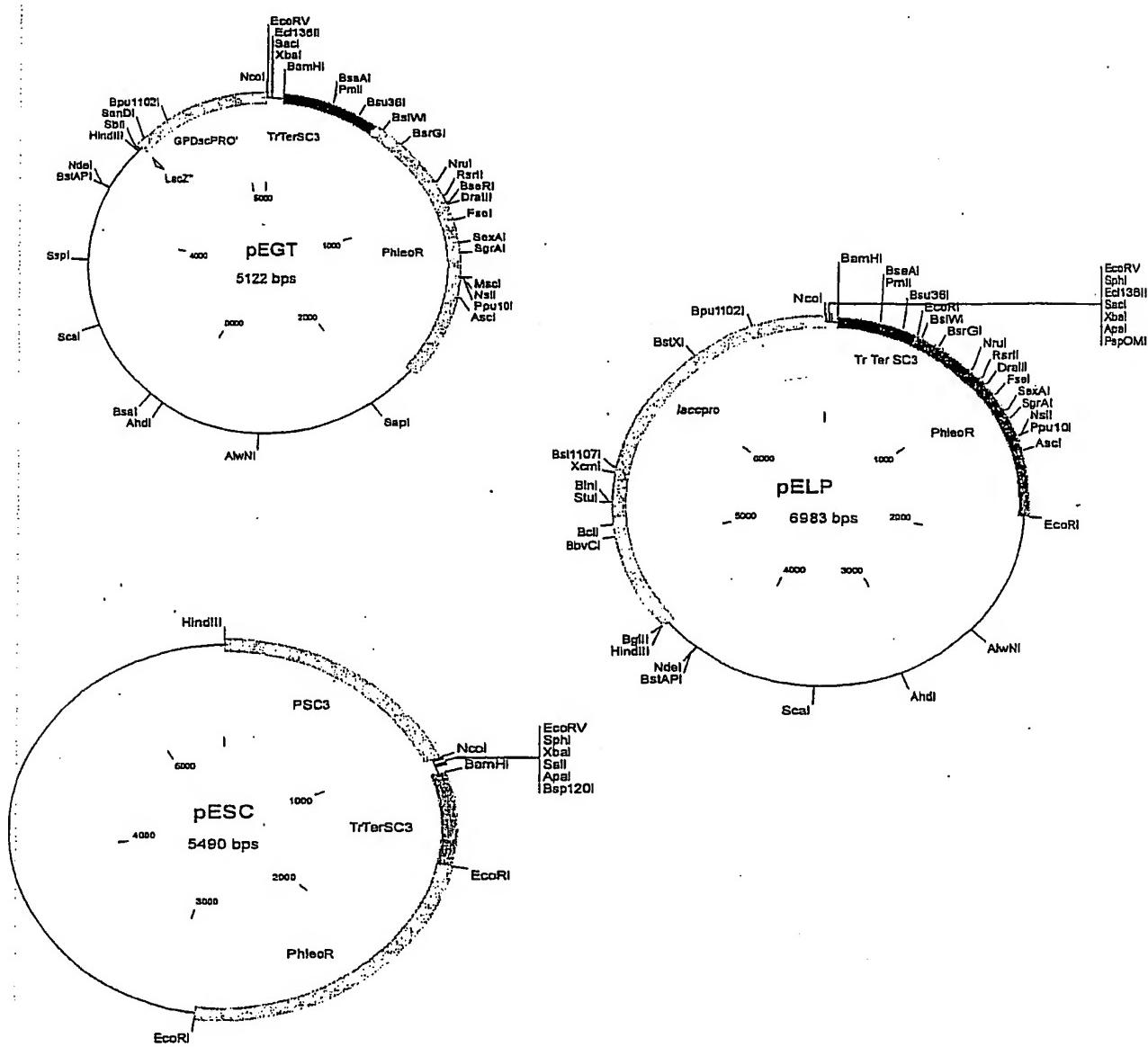


Figure 6 : Carte physique des trois vecteurs d'expression utilisés pour la production de la laccase chez *Pycnoporus cinnabarinus*

CATGGGATA TCGCATGCCCTGCAGAGCTCTAGAGTCGACGGGCCCCGTACCGCGCCGCC TAA GACGCCCTGGATCCGCAGGTGAAC
GCGCCTATCGGTGGGATA TTGGCGACGGGAGCCTCGCAACTCGAGCCCTGTTACTGCCCTAGCAAATCGGAATCCCTCGATGT
CATAGGGTGC CGGACAAGTGATCGTCTTGCATACACTCCAAGGTGTTGACTCATTCCCTCGATAATGAACATTTGTTGTTGTTG
TTCTCTATCGCTCAGTCACCGAACCCCACACGTGATGGTGAACTTCCGCCACGCCAACAACCGCATGACGACATGGCAACCTAAG
TAAAGGCTGAGTCGTTGACTAAAGCAGTCCACTTACGGCGAGGATGCCAGTCTACGTCATGAATGAAGGCTCAGGTCCCGAAGTAA
GGGGGTACAAAAGGAGGGTGAAGGTGGACGTTTCTTACCATCTTCCACCTCCAGAACCAACCATGCCGGGAAATTCCCGAGCTTGT
CAAAAAGGTTCTGCCGTACGCCCGAATTCCTCGAGGTGGCCCTATCGCATACATGCCAGACTTCAAAACATCCATTCTATC
ATTTGGGATCGTACAATTAGACATGTTGACAACTTACATTCTTCTTACTCTCCGGCCAGTCTATGTAGAGGTAAA
GTACAAGCGTCAAAGGATCAGGCACTTAGAGCGCGCGTCTGCTCGCCGTTAGAGCGCGCCGCTCTGCTCGCCGCTAGACG
AGCAGGTGCGAGACACGGCGGGAGTAGCCCAACTCGTGTGCTACCAGGCAATGAGCTTACGAAGGCTCAGAAGCTTGTGATCGGATGCCG
GGGATCGATCCACGGCTTAAAGCGGGCCGGTACCCCGTCCGAGCCGCTGGGCGCTGGGAGCGGCGGTGTTGGTGTGGCGTCCG
TCAGTCTGCTCTGCCACGAAGTGCACCGCAGTGGCCGGGGTGTGGCAGGGCAACTCCGCCACCGGCTGCTGCCGAT
CTCGGTATGGCGGGCCCGAGGGCGTCCCGAAGGTTGTCGGCACCCACTGGTCTGGACCCGCTGATGAACAGGGTACGTGCTCCGGCAC
CCACACCCAGGCCAGGGTGTGTCGGCACCCACTGGTCTGGACCCGCTGATGAACAGGGTACGTGCTCCGGACCACACCGG
GAAGTCTGCTCTGCCACGAAGTCCCGGGAGAACCCGAGCCGGTCTGTCAGAACTGACCGCTCCGGGAGCTGCGCGCCGGTGA
CCGGAAACGGCACTGGTCAACTGGCCATGCACTGGTGTGGGATGGCATTATGTGTGATGGGATGCCATGGGAGAGGGAAAGTGTCTGGATG
GGAGTGTGAGAAAAGGGAGACGGCGGGCGCCCTGGACTGCGGCCATCTGCAAATGCCAGGCCAAGATGCCATGCAACTGACAAAAGGGA
TGAACACATCGCGGGCGCCCTGGACTGCGGCCATCTGCAAATGCCAGGCCACTGGCTCGGCTGGGCGACCACAGGCCCTGGCTGAGT
CCCCCTGAGGGCGACGCTTATCTATCCATGCGCGCAATTGCAAGGTGCGCGGTGCAAGAACAGTCTCTGCACTGCCCTCGCACC
TGGGCTGGCACCCCTGTCTACCTCTCATCTAACCCCTCCGGCTTCCGAGTACAGTTACTAATCTCACACCGAAGAGGGCTCGC
CACCCCTCGATCCCAGACGCTTCTACATGCCACAGCGTCAAGAATGAAACAAATGCACTCARATCAGATCCCCGGAAATCGT
AAATCATGGTATAGCTGTTCTCTGTGAAATTGTTATCGCTCACAAATTCCACACAACTACAGGAGCGGAAAGCATAAAGTGTAAAG
CCTGGGGTGCCTAATGAGTGAAGTCAACTCACATTAAATTGCTGCTGCGCTACTGCCGCTTCCCTGGGAAACCTGCTGCGTCC
GCATTAATGAATCGGCCAACCGCGGGGAGAGGGCGTTGCTATTGGGCGCTTCCGCTCTCGCTCACTGACTCGCTGCGCTCG
GTCGTTGGCTGCGCGAGCGGTATAGCTCACTCAAAGGCGTAATACGGTATCCACAGAATCAGGGATAACGCAAGGAAAGA
CATGTGAGCAAAGGCCAGCAAAGGCCAGGAACCGTAAAAGGCCGCGTGTGGCTTCCATAGGCTCCGCCCTGAG
AGCATCACAAAATCGCCTCAAGTCAGAGGTGGCGAACCCGACAGGACTATAAGATAACCAGGCGTTCCTCCCTGGAAGCTCC
CTCGTGCCTCTCTGCTTCCGACCGTCCGCTTACCGGATACCTGTCCGCTTCTCCCTCGGGAAGCGTGGCGCTTCTCATAGCTC
ACGCTGAGGTATCTCAGTGGTGTAGGTGCTCAGGCTGCTCAAGCTGGGCTGTGTCAGCAAGGCGCTTCAAGGCGTTCAG
TTATCCGTAATATCGTCTTGAGTCCAACCCGTAAGACACGACTTATGCCACTGGCAGCAGCACTGGTAAACAGGATTAGCAGA
GCGAGGTATGTAGGGCTTACAGAGTTCTGAAGTGGGCTAACTACGGCTACACTAGAAGGGACAGTATTGGTATCTGCGCT
CTGCTGAAGCGAGTACCTTCCGAAAAAGAGTTGGTAGCTGCTGATCGGCAAACAAAACCACCGCTGGTAGCGGTGGTTTTGTT
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ACAGGCATCGTGGTGTGCTACGCTGGCTTGGTATGGCTTACCTACGCTCCGGTCCCAACGATCAAGGCAGGTTACATGATCCCCCA
TGTGTGCAAAAAGCGGTTAGCTCTCCGGTCTCGATCGTTGCAAGAAGTGGGGCAGTGTATCACTCTGGTATGGT
AGCACTGATAATTCTTACTGTCATGCCATCGCAAGATGCTTCTGTGACTGGTAGACTCAACCAAGTCAATTCTGAGTATGG
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TCITCAGCATCTTACTTACCCGACCGGTTCTGGGAGCAAAACAGGAAGGCAAAATGCCCAAAAAGGGATAAGGGC
ACCGAAATGTGATACTCAACTCTCTTCAATTATTAGAGCATTATCAGGGTTATGTCATGAGCGGATACATATTG
AATGTATTAGAAAATAACAAATAGGGTTCCGCGCACATTCCCGAAAAGTGCCTACCTGAGCTAAGAAAACCATATTATCA
TGACATTAAACCTATAAAAATAGCGTATCAGGAGGCCCTTCTGCTCGCGTTCTGCTGATGACGGTGAAAACCTGACATGC
AGCTCCCCGGAGACCGTACAGCTGCTGTAAGCGGATGCCGGAGCAGACAAGCCGTCAAGGGCGCTAGCGGGTGTGGCGGG
TGTGGGGCTGGCTTAAACTATGCCATCAGGACAGGAGTGTACTGAGAGTGTGACCCATATGCCGCTGGGACCCAAMTA
AGGAGAAAATACCGCATCGGCCAACCGGCCATTGCCATTAGGCTGCCAATGTTGGGAGGGCGATCGGTGCGGGCTCTCGCTT
CGCCAGCTGGCGAACGGGGAGTGTGCTGCAAGCGATAAGTGGGTAACGCCAGGGTTTCCCTGAGCTGAGCTGTTGAAAACGAC
GGCGCAGTGCCTACGCTGCTGAGGTGCGACGCCAGCGCGCCGCCACCCAGCTATCCCGCGCGGGTGGGACCCAAMTA
GCCGGCCCCCGCGGCCCGGGCTGGCGAGCGGGGTGATCTACGAACGGAACCTGGAGGGCGACTCGGAAGAGTTGGTAAAGGG
GAACACCATCGCGAACGGGCCAGTGTCTGDCAGCTGAGCGTGCATTGTGTTCAATTCTGACCTGTGGCATGTAAGGAACGTGCTC
GGGATCGGAGGGTGGCGAGGCCCTTCTGGTGTGAGATTAGTAACTGACTGCGAAGCCGGGAGGGGGTAGGATGAGAGGTAG
ACAGGGTGCAGGCCAGGTGCGAGAAGGACTGCGAAGGACTGCTCTGCCGCCACGCCAATTGCGCGCATGGATAGAATAGA
GCGTGCCTCGAGGGGGACTCGACCAAGGGCTGGTGGTGGCGGCCAGGGACTGGCTGGGCAATTGCGATGGGATAGAATAGA
GCCGCCGCCGATGTGTTCATCCGTTGTCAGTATCGATGGATCTTCGGGCTGGGTTATAAAAGCGCAGCGCCGCCGCTCC
CTTCTCCAGCACTCCCATCCAGACACTTCCCTCTCCATCGCATCCCCATCACACAAATAATGCCCATCAC

Figure 7 : Séquence nucléotidique du vecteur pEGT, contenant le promoteur du gène gpd (4480-5122), un marqueur de résistance à la phléomycine (507-1822) et le terminateur du gène sc3 (71-507).

AGCTTCTCGCCCCGAACTGAACGGCAGGATGTGGGGCGTCCAATATTGCCATGAAAATCTGTCAGAAGTGAGCCCTCTCGTCA.C
CCTGTACAGCTCGTCACTGAGTTGAAAAGCAGGGTICATCTGGCTCACTGATGCACTGAGCTCGACCCGAGAACTAAATGACCAGCCGG
AGTGTCACTAACTTAACGCCGGTATTCAAGGGCAGCTTCTATACTGTCAGCGTAGATCACCGCCCATGAACGGGGAAACG
GGGAGGGGTCGTTGGTACGCTTACGCTCTGGCTATGTTGATTGACCAAGCGTCTGCAGAAGATGGGACGACGATGCCGAGCCG
GCCAGTGTCTCGGATGTCCTACTGTTGAGGCCATCCTTGTAGACAGACGGAAGAGCTTGAGGGTGCAGTCCCTCTAGAATGGGA
AGGGGCTTAACTGAGATGGAGAGATGACACGCTCTGAGCTCCCAACAGCCTTCGCCGAGGGTGCCTCCGGACATTCACTCAGTTCTAGG
TCTGACCTGCTTAATTGATAGACGGGCCAACAAACCTCTGTCAGGCCATCATAAACAGTGCCTGACAGAGCCTTCCACTCAGTCGG
CGCCTCCCTCAATCAATCCCCTAACTCGCCGGCTCTGCCCTTCGCCGTCAGACCGCTTGAAGAGGCCGACGGCGTCCGC
TCCCCCCTCCCTCGCTGTCATGCACCGCAGCGTAAATGTTGCTGAGGGAGCCGTAAGTATATTCAAAGGCAGCGCAATGAATAG
CAGGCGCGGGGACCTGGCACGCGGGCATGAACATGCAGACTTGGGTGACGATAACTTGAACCTCAGACGCCGGAATGAATATCCA
AACCGCGGGQAAGAAAATAATTACCGGAGCCTCCCAAGGTATAAAAGCCCTCACCGCTCAGTCTCCAGTCAAGCAGCCAGT
TCAACTACCCAGCCCTCTCTCTGCTATCCTCYTTACAACCTGTCGCCATGGGATATCGCATGCTGCAAGAGCTAGTCGAC
GGGCCCCGTTACCGGCCGCCCTAAAGCCTGGTGGATCCGAGGTAAACGCCCTATCGGTGGATAATTGGGGACGGGAGGCTCGC
AACTGAGCTCTGTTACTGCTTAGCAAATTGGAATCCCTCGATGTCTAGGGTCCGGACAAGTACGCTCTGCTACATACTCCAAG
GTGTTGACTCATTCCTCGATAATGAACATTGTTGTTGTTGTTCTATCCGCTCAGTCACCGCAGCCACACGTGCACTGTTGAAC
TTGCCACGCAACAACCCATGACGACATGGCAACCTAAAGTAAAGGCTGAGTCGTTGACTAAAGCACTCCACTTACGGCAGGGATG
CAGTCTACGTCATGAATGAAGCCTCAAGGTCGGAGTAAGGGGATACAAAAGGAGGGTGAAGGGTGGACGTTTCTTACATCCCTCCA
CTCCTCCAGACTTCAAAACATCCATTCTATATTGGGATCGTACAATTAGCATGTTGACAACGTTACATTCTTCTTCT
TTACTCTCCGGCCCACTGCTATGAGAGGTTAAAGTACAAGCGCTTAAAGGATCAGGCACTTAGAGCGCGCCGCTTGTCTCCGCTTAG
AGCGCGCGTCTGCTTCCGCGTAGACGAGCAGGTCGAGACAGGGGGAGTAGCCCACTCGTTGCTGACCAAGGCAATGAGCTT
CACGAAGCTCTGCTGATCGGATGCCGGGATGATCCACCGCTTAAGGCGGCCGCGTACCCCTCGGACCCGTCGGCCGCGTC
GGACCGGGTGTGGTGGCGCTGGTCACTGCTCTCCGCAAGCAAGTGCACCGCAGTGGCGCCGGGAGGGCGACGGGAACTC
CCGCCCCAACGGCTGCTCCGCGATCTGGTCTATGGGCCGGGGGGAGGGCTCCCGGAAGTTCGTTGACACGACCTCCGACCCACTCGCGT
ACAGCTCGTCAGGGCGCCACCCACCCAGGGTGTGCTGCGACCCACTGGTCTGACAGTCTCCGGAGAACCCGAGCCGGTCCAGA
TCGTCGGGACCAACACCGGAGTCGCTCTCCACGAAGTCCGGAGAACCCGAGCCGGTCCAGAACTCGACCGCTCCGGCGAC
GTCGCGCGGGTGGAGCACCAGAACGGCACTGGTCAACTGCCATGATGGTATGGGATTATGTTGATGGGAGAG
GGAAGTGTCTGGATGGGAGTGCTGGAGAAAGAGGGAGACGGCGGGCGCCTTTATACCCACGCCGAAAGATCGATCGATA
CTGACAAAACGGGATGAACACATGGCGCGGCCCTGGACTCGCCTGCACTGCAAATGCCAGCGCAGTCCCGTCCGGGCCACCCACCA
GCCCTGGTCAGTCCCCCTCGAGGGCGACGCTCTATCTATCCATGCGCAGGATTCGAGGTGCGCTGCAAGAACAGTCTCGCAGT
CTCTCTCGCACCTGGGCTGCACTGCTCTCATCTTAACCCCTCCGCGCTTGTCACTGAGTACTAAATCTCACCGGAAGAG
GCTCTCGCGCACCCCTCGATCCCGAGCACGTTCTACATGCCACAGCGTCAAGAATTGAACACAATGACGTCARATCAGATCCCCGG
GAATTGTAATCATGGTCAAGCTTTCTGTGAAATTGTTATCCGCTCACAAATTCCACACAACATCAGAGCCGGAACGATAAAAGTG
TAAAGCCTGGGTGCCCTAAAGTGAAGCTCAACTCACATTAAATGCGTCTGCGCTACTGCCGCTTCCCTCGGGAAACCTGCTG
GCTGCACTTAATGAATGCCAACGGCGGGGGAGGGGGTGTGCGTATTTGGCGCTTCCCTCGCTACTGACTGCTGCGCT
GGTCTGCTGGGTGCGCGAGGGTGTAGCTGCTTCCGCTCAAGCTGGGGGGTGTGCGTACGGCAACCCCGCTGCGCCCTGGGTA
ACTATCGTCTGAGTCCAACCCGTAAGACACGACTTATGCCACCTGCGCAGGCCACTGGTACAGGATTAGCAGAGCAGGGTGT
GGCGGTGCTACAGAGTTGAGTGGGCTTAACTACGGTACACTAGAGGAGCAGTATTGTTATCTGCTCTGCTGAGGCCAGTT
ACCTCGGAAAAAGAGTTGGTAGCTTGTATCCGCAACAAACCAACCGCTGGTAGGGTGGTTTTGTTGCAAGCAGCAGATTACG
CCGAGAAAAAGGATCTCAAGAAGATCTTGTATCTTCTACGGGCTGACGCTCAGTGGAACGAAAACCTCAGTTAAGGGATT
GGTCATGAGATTATCAAAGGATCTCACCTAGATCTTTAAATTAAAGTAACTCTAAAGTATAATGAGTAAAC
TTGGTCTGACAGTTACCAATGCTTAACAGTGGACCTATCTCAGCGATCTGTCTATTGTTCACTCAGGTTACAGGATTAGCAGAG
GTGCTGAGATAACTACGAGGGCTTACCATCTGGCCCTAGTGTGCAATGATACCGCAGACCCACGCTCACGGCTCAGGCTCAGG
ATCAGCAATAACCGCAGCCGGAGGGCCGAGCGCAGAACGGTCTGCACTTTATCCGCTTACAGCTATTAAATTGTTGCC
GGGAAGCTAGAGTAAGTAGTCGCAAGTAAAGTGGCTGCACTGAGGAGTACATGATCCCCATGGTGTGCAAGGGTGTGCTG
GTATGGCTCATTCACTGGCTCCGAGGGTGTGAGCTGAGGAGTACATGATCTCTGAGAATAGTGTGCTGAGGAGTGGCTT
CTCCGATCGTGTGCTAGAGTAAGTAGTCGCTCCGAGGGTGTGAGCTGAGGAGTACATGATCTCTGAGGAGTGGCTT
AAGATGCTTCTGACTGGTGTGAGTACTCAACCAAGTCTGAGGAGTACATGATCTCTGAGAATAGTGTGCTGAGGAGTGGCTT
ACCGGATAATACCGCAGCCACATGCAAGAACCTTAAAGTGTCTGAGGAGTACATGATCTCTGAGGAGTGGCTT
CCGCTGAGGATTCAGTCTGAGTAAACCAACTCGTGCACCCACTGATCTCAGCATCTTACTTCACTGCTTCTGAGGAG
AAACAGGAAGGAAAATGCCCAAAAAAGGGATAAGGGCAGACGGAAATGTTGAATACTCATACTCTCTTCAATTATTG
AGCATTATCAGGGTATTGTCATGAGCGGATACATATTGAATGTTAGAAAAAATAAACAAATAGGGGTTCCGCGACATTTCCC
CGAAAATGCCCCCTGAGCTCAAGAACCTTAACTTATGACATTAACCTAATAAAATAGGGTATCACGGAGGGCTTCTGCTCG
GGCTTCTGGTGTAGCGGTGAAACCTCTGACACATGCACTGCTCCGGAGACGGTCAAGCTGCTGAGTGGCTT
AAGGGCTAGGGCGCTGAGGGTGTGCTGGGGCTGGCTTAACTATGCGGATCACGGAGATGAGACTGAC
CATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCCATTGCCATTGCGCACTGTTGGGA
AGGGCGATCGGTGCGGGCTTCTGCTATTACGCCAGTGGCAAAGGGGGATGTGCTGCAAGGGATAAGTGGGAAAGCCAGGGT
TTCCCACTGACGAGCTTGTAAAACGACGGCCAGTGCCTA

Figure 8 : Séquence nucléotidique du vecteur pESC, contenant le promoteur du gène sc3 (1-1033), un marqueur de résistance à la phléomycine (1540-2855) et le terminateur du gène sc3 (1104-1540)

Figure 9 : Séquence nucléotidique du vecteur pELP, contenant le promoteur du gène laccase (4457-6983), un marqueur de résistance à la phléomycine (507-1822) et le terminateur du gène sc3 (71-507) (suite de la séquence, page suivante)

CAGCGAAGGTCCGTTGCCGATGGGAAGCAGGCAATCAGTGGGTGCTTACGCCGCCACGATGTCGGGGAGCGTAGGCGCCCTCCCA
TAAGCGGCAAGCATCATGATGCTCTCGATTCCGATTGGAGACTTAAAGTGAGTGAGAAGGGCAGCAGAGACTCTCCGAGAGACCAGTGTGCGCAC
GTTCCCTGGCTTGGAAAGACTTAAAGTGAGTGAGAAGGGCAGCAGAGACTCTCCGAGAGACCAGTGTGCGCAC
CTGGGAAGGATGGCTCTGGTAGACATTGGCGAAGGTGCTTAGATGTCAGCGGGCTTCTGGATGATCATGCTAACCTTCTGA
CCTCGTCGGTGGTACGCATGGCAGGATTGAGCATTACGGTATGCCCTCCATTCAAAACGATAACCCCTCCCTCAGGTTGGTCACTC
CATAGAGCGGCACGCTCTCAAGGCTAGGCTTACACACCTCTCGCAACATCCCTATTACGGTGTCTGAAGGAACGACTTGTCA
GGGATCACATGAAGTGAGCATACTGTCGCCGGTCTCGCAGTACAGACGCTAGTACGGGAAGTCGACATCCAAGCGTTAGTCACCA
CATGGCAAAAAGCTGCACCAACTCTTATGGTAGTTGAGTTGCTGAGTGGTATACAGTCATTATCGACGGGAATGCCACCGGATAGG
GTGTGGCGGCCAATATTACATGCCCTGGCAATAGTCGATGTCGCTCTTGTCAATGAATATCAGGGTCAATGTGGAGACGGTTAA
ACAGCGTGAAGTGTGAATCCCTGGTGTGTTGGCGAACAGGTACGGTCAAGGAACACCAATATCTCTCGCAGCCCAGTTCTTG
CGAGCGGCACAGGCAGGCATCGCGAACAGATCCCAGCATCCGGCTCTGACATTGGGATACCTGAAGCCCTCAGGTACGGAGC
GAAGAGGTGGCTCTCGCAGCGATTGGCGAACGGGATAGCTGTTTCTCTCACCATTGGGAAGATGTGAAAGGCTCCATCATAT
AGCGGCTCAACTCTACCTCGAACATGTCACACGGCGGGAAACTTATTTATGTGACAAGGCCGAGCTATGATAGCTGCTCCCGAA
GTGGTAAGTCCCACATCGCGGTTACGGCAACAGTCTCGGAAAAAAATAAGAAGAATATTGTAGGTGCGTGTAGGCATCGCCCCAAA
TGCACACACGGAGGTTAGGAGATGAAGCGCCCGTGAAGCGTAAGGGAGTTGGTACCGCGCCCCGACCGACTCTCTCTT
CCCAGCATCATGTCGGCGAACACTTACCCCTATTGACCAACTCCACGAGAAAGCAGGAACAGCTTCTTGTCTCATGACGCC
GCAATCCAGACCCCTAGCCGGTTCTGTTACTCATCGTTATCCCTGCCCATCGTAGGGAGTCAGGCTGGCAGTGCCTAGTCCCGTCT
CTCTGCTGCACTAGAGAAGCCCCATGAGACAGCGTTTCTGCTTATTCCTGCTGTTCTATAGACACCATAGGGCAAAACGATCCCTG
CACGCCAGAGGTATTGGCTCGTAGATCCCAGTTCTCTCGGCTCTGAATCGGCTGCACGGCAGATAATGGGCCGGAAATGCT
ATAGGCTTCAAGCCCCCTATGAGAGTGCACAAAGGCTTGTCAAGTCAGGCTGGCTCTCACGAAGAGCGTCAACTCG
CGACAGCCCTTCAAGGGCAAGATAGATCTCCCATCATCCCTACTGCGCTCAGCGCCGGTACCGAACAAATTGACTTACCGACATC
CTCCCGACCGCAAATGCTGTTGACGGAACGTAATCTCTCGTCCCGCTCTTCTGCTCTCACGCATCCGTGTTGCGCGCA
CGGCCGCTCATCAGGACCAAGACCACTCAATGTCGGTACCGGCACAAATGGTACACTGCGGCAACTGAGTAGGTCTGTCACTCTG
GTGCAACCGTCGTTACCGCTGACCTTCGGGATACTGTCCTGCAGACATCTGGAGCGCTGTTTCCCTAGTATAATGATGTCTGTCC
GCAGGTCTTGAAGACCGCTGAGTCCCACCTGAGTTTGTAGGTAGGACCTGTTCTCCACAAACCCCTCTT

**Figure 9 : Séquence nucléotidique du vecteur pELP (suite),
contenant le promoteur du gène laccase (4457-6983), un
marqueur de résistance à la phléomycine (507-1822) et le
terminateur du gène sc3 (71-507)**

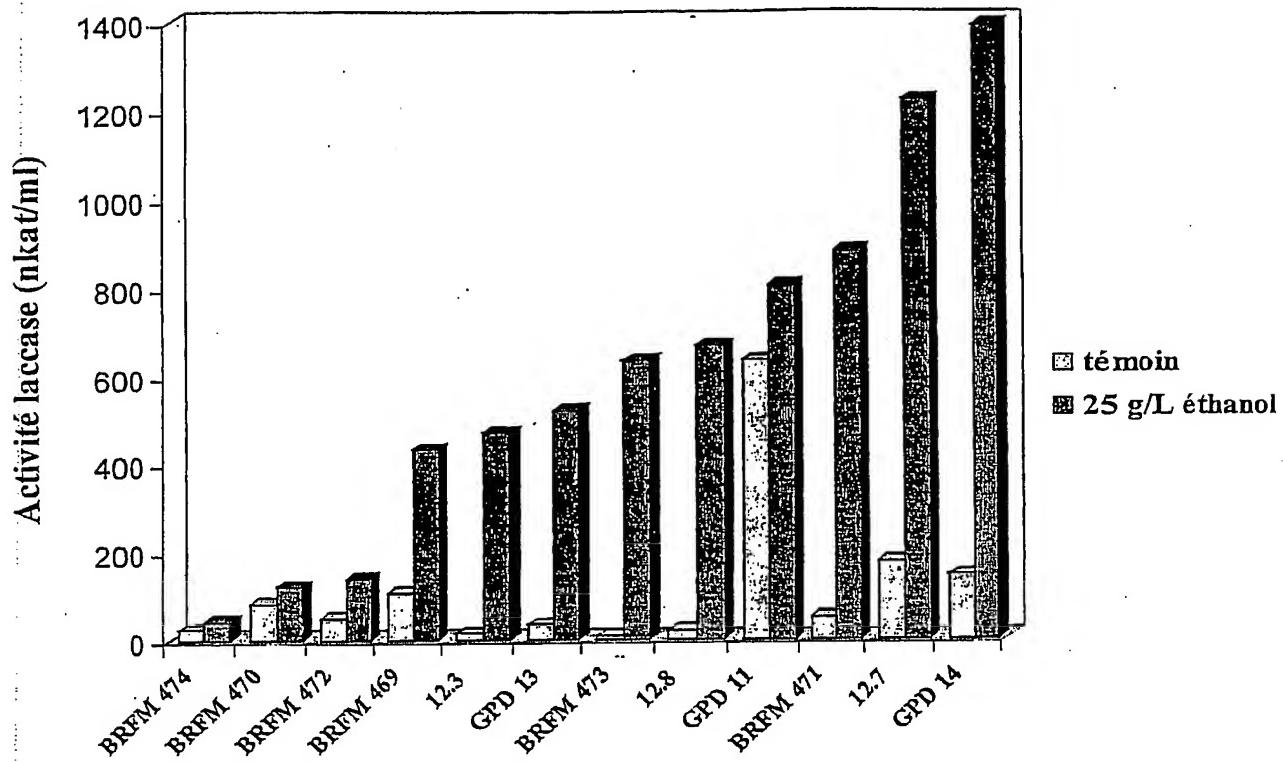


Figure 10 : Résultats de production des transformants présentant les activités les plus importantes. La culture a été effectuée avec ou sans (témoin) éthanol

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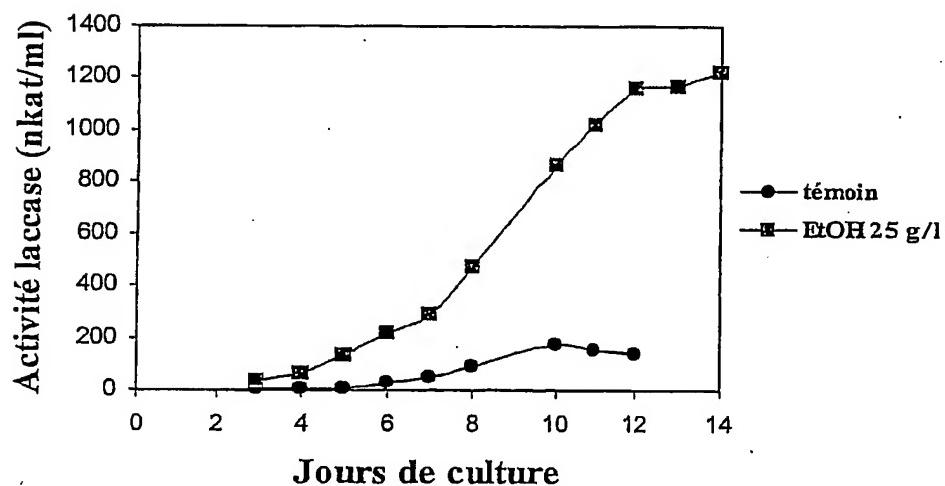
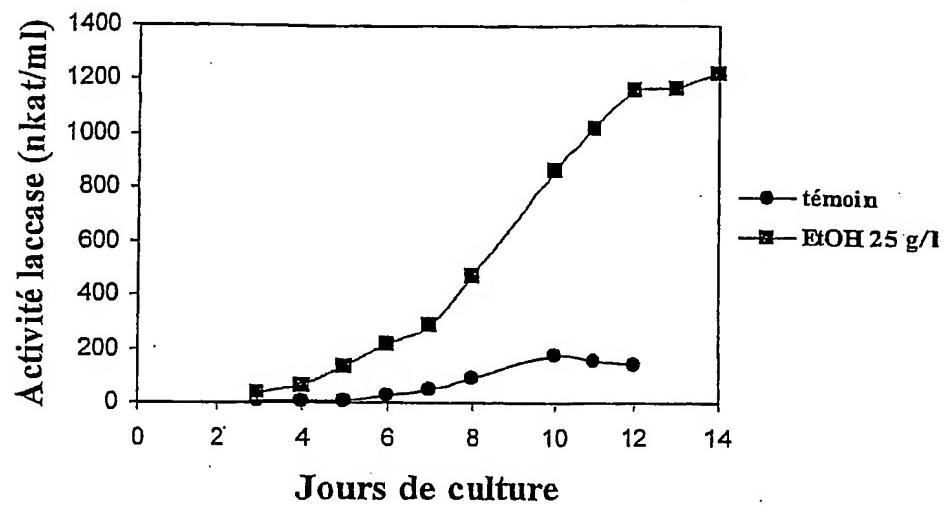


Figure 11 : Suivi des activités laccase des transformants GPD 14 et 12.7 en fonction du temps avec ou (témoin) sans éthanol

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TGGGGAGATGGTCTATAATACAAATGTCCTCTGCCCTGAGCTTCCCGCCTGGTGTCTGTCATGTCAGTGCGCGCACATGCCTTATTAAACCGT 100
 TGGGGAGCTGCCCGCGCCCAAGGAGATAGCATAATCGCTGAGAACCTAGTCGTCATGGCGGTGTAACCGTTCTGGCATTTATTTCGCACCTTC 200
 TCAAGAATATAAAGGCTATGTGATACGGTTCATCTAACCCCAGCGTCCCTCCGAAGAGGGCGTCCTCTCACTTCGCACTTCTACTGCTTAA 300
 CTCAGTTCTAGCGCGCTGTGGTCCCGTGTACGGACTTAAACACTGTCAGTAGATACTGTCGCCCGCGACGGGTGCTGCTTCTGGCGGGAGGGACTTGT 400
 S V H A A V G P V T D L T L I V D T V A F D G A A F R E 43
 CGACTGTAATGCCGGATTGGATTTCTAATTATAATCTCCAGGCAATTGTCGTCAGAGGAAACCACACTCCGTCATGGTCCGGTCATCGT AGGGTGGG 500
 A I V V Q B E P N S V I G P V I V 60
 TAGCTACRGAGTCTCTCCCTCATTTAGCTCATCACCAAGTGTATGATATTATAAGGTCAGAAAGGGGGACACTTCGCGTCATGTTTACACAT 600
 L D S P N M R Q S T S I H N H G I F O G N 74
 TTGGATTCTCGCAACATGCCCAACTCTACTTCATTCATGGCATGGCATCTTCCAAGGAAAGCGTACGGTGTATATCGGATAATCTATCTGTATCCATT 700
 GACTCGGATATAGGTCAAGGATTGGCGTGGCTGGCCCTCCTGAAGCCGTCGTCGAAATTATCTTCCTGAAATTTTAGATGGCGCCGCAATTCGTTAAC 800
 G Q N W A 95
 AGGTAAGGAGATGTCCTCCCTCGTTCCCCAGAACTAATTATCTCTAGTGGCCCAATTGCCCCCGGAGGGACTCGGTTCTGTACGACTTACCGAACCT 900
 Q 107
 TTCCAGACTGGCACATTTGGTATCAITCCATTATCAACTCAATCTGCGATGGACTGGGGAGCATTCGTCGTTCTCTCTCATCAAGTC 1000
 F Q T G T F N Y R S H L S T Q Y C D G L R G A F V 125
 CCGCTTCTCTCACTTATCTAGATCTACGATCCGCTCGACCCCTTACCGGTTGCTCTAGATGTCGACGRCGAGTCGCGCTGTGATTACTCTGGCGGACTG 1100
 I Y D F L D P Y R L L D V D D E S T V I T L A D W 176
 GTACCCACAGCTATGCCGAGGACATTCATCGCCTAGGAGATTTCCCAGATGTCTCCCTGCCTCTGTGAATTCAGACTAGTGAGGC 1200
 Y H S Y A E D I L I A 191
 TCTCTCTCATGTCACGGGAGATTGGCGGAGCCGGCGGAGAACGGCAACAGAACTATCTGTCATTACTGTTGAGCATGGAAAGCGGTAGGGCATTC 1300
 I L I N G H G R F A G A G G T A T E L S V I T V E H G K R 220
 CGGCTTGTAGATGTGTCATTTGTGATAGCTACCGATTTGGCCATTATCGCCTGTCGACCCCTGGTTTGGCGTGAARATCGATGCCATACGAA 1400
 Y R L R F A N I A C D P W F A V K I D S H T N 243
 CCTTCGCGTATCGAAGCTGACGGTATTACTACTGTGCGCTGTACGGTGGACTCCTCAATGTAAGGTTACCCCTAGCACTTCCCACCTCTGGATCCT 1500
 L R V I L E A D G I T T 254
 TATGACTTCCCAAGTCTTGTGGCCPACGATATGTCATCTCCATGCCAACCCGCTGTTGGAAGACTACTGTAAGCTGCTAAATGTTGCATGAC 1600
 T 1 F V G Q R Y S V I L H A N Q F V G N Y 274
 TGTCATGATTCTAACCCCGCAGGGATTCGGGCGCGTCCGACACGCGTGGGGATCTGACTCGGCTATTCTCGGTTATGTTGGCG 1700
 W I R A A P N G V S N F A G G I D S A I L R Y V G A 300
 CCCAGAAGAGAGGCCACACTAGTGGAGATACTCCATGCCACACACTCAAGAGCAGGATCTTCACCCGCTGATCTACCCGGCGCCAGGCATCCAC 1800
 P E E P N T S E D T P B D T L Q E Q D L H P L I L P G A G I H 333
 TCCCGTGGGGCCCGGACGTTGCCACACGCTATGAGTTCTGACTGTGGCGACTTTCTGGCCCCCTTATTATATATCTGGTTAGGATGCC 1900
 S R G A A D V W H T V S M E F 348
 GCAACATTCCAAATCTCTCTGGATGGCGTGGCCCTCCAGCCGTGGCTCATCTCTTCACAAGGATTTATCTAGCTGACGATTTGAATGTAGCC 2000
 L T L I K C S F T 357
 TGCCCGTCCTCTGCCATTTATCGGGAGCCAGACTGCTAATACCCCTCTCCGGGGATCTTCTGACTCCAGCGTCCGACATGACATCGTGGAGCT 2100
 M P V L L Q I L S G A Q T A N T L L P R G S F I Q A S H N D I V E L 391
 CAATTTCGGCAGCTTCGCACTGGCGTGGCCATTCTTCCTGCGAGCTGAAATTACTGCTCTTCTGACATCCAACTCCAA 2200
 N F P A V N V A A V G G P 409
 GTGAGCGCAGCGGGACCTTGGCTATGGCATATGACTTATTATAGCCATGGCCATGCACTGGCTTACGCTCTGTTGGAAAGCAACCTGCATAATC 2300
 H G H A F D V I R S A G T N S D N 426
 GGTCAATCGGTATTCTTCATTCGACTTCCATAGATGACGATGGCTCACTATGGTTTACCCAGGCTCGAGAGATGTGCTATCCACCGGTACCGATC 2400
 W F N P 441
 CTAATGACAACTGTGACGTGTTTGGCTATGATGTCCCTTGTGTTGGAGAGCTACGTTCTGGCGTGGCGTAAACCCGATCGCTTAACTG 2500
 P N D N V T I R F R A D N P 455
 CTGAATCTCTGTTGCTTGGCTCTCATATCTCATCAGGGTCCATGGTCCTTCACGCCACATTGACTGGCACCTGAACTCGGCTTGTGCTTGG 2600
 G P W F L H C R I D W H L E L G F A L V 475
 GATTGCGAGCGCCTAGCGATGGGACAGCGCAATTAAACCTCCTGGTGGCGTGCCTGTCAGATGACACATTCGCTTAACTGCTAGCTGCT 2700
 I A E A P S E H D S D I N P P A 491
 CGTGGGATGACCTATGCCCTACGTTGGCTGGCTCTCTTACTTATCTCAAGTTCTCACTTCACAGATATGACCCCTGGCGCGCT 2800
 A W H D D L C P T A W L L F Y Y F R F B P H I L N E T D M M P C R L S 525
 CAGCAGTAACTGAGCTTAAAGACCTCAAGGTTGACTAAGGAAAAGCGAAGCAGTATGAACTCTCATTTATCTTATATCGACACATTCAATTC 2900
 S S R N V K N L N V D 536
 CCTACGGGTTTCTCCGCGACCTGAATTGGGTGCTAGATCCCACATCTGGTGGAGTAGGAAAGAAATTCTGTATAAAACCCATGGGTCTCT 3000
 ARATATACATAACGTCGCGGGTAGTTACGCT 3037

Gène de la laccase d'*Halocyphina villosa*

Figure 12

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